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RESEARCH ARTICLE

# European brown hare (*Lepus europaeus* Pall.) testes weights from different seasons and ages from Bulgaria

Chavdar Zhelev[1,2], Nino Ninov[3]

[1] *Southwest State Forest Enterprise, Blagoevgrad, Bulgaria*
[2] *Forest Research Institute – Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *University of Forestry, Sofia, Bulgaria*

Corresponding author: Chavdar Zhelev (chavdar.d.zhelev@gmail.com)

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## Abstract

The testes weights of 315 European brown hare from different ages were measured during a whole year in Bulgaria. The results indicate that the active reproductive season finishes at end of August and the beginning of September, whereupon follows a period of calm when the testes weights are at their lowest. In September, October, and the beginning of November, the weights of the testes are low. The first indications for the start of the new reproductive season were shown in November, with the gradual growth of the testes weights. In January the testes weights reached high levels, as the highest weights were measured in May-June, with slightly lower values in July and August. Low weights followed after this, when the calm period started in September and the testes growth is reactive in December. All adult males (above 1 year old, determined by dry eye lens weights, n = 160), had high significant values ($p<0.0001$) of testes weights during the whole year, compared to all the young ones (under 1 year old, determined by dry eye lens weights, n = 155). The results showed a normal reproductive cycle with periods of activity and calm in male European brown hares from Bulgaria. The distribution of testes weights, compared to the various ages, follows the same sequence, as the sperm density (we assigned sperm quantity for each individual in the epidydimides to five classes: 1 – no sperm, 2 – very little, 3 – moderately present, 4 – high amount, 5 – massive amount). In view of the fact that the density of sperm is defined by the tail of the epididymis, and the weights were measured from the testes, the connection between them proves the existence of a normal reproductive cycle with stages of activity and standstill. The heaviest testes had the biggest quantity of sperm in the epididymis, regardless of the season. We determined that the quantity of sperm and the weights of the testes were in direct dependency ($p < 0.0001$), directly following the line of activity and standstill in the seasons.

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**Keywords**
testis weight, brown hare, sperm density, testicular activity

## Introduction

The last 3-4 decades saw a decline in the brown hare population density in the places, where it occurs on the European continent. Some authors (Tapper, Parsons, 1984; Hutchings, Harris, 1996; Marboutin et al., 2003; Smith et al., 2005) state various reasons (change in habitats, intensive agriculture, reproductive problems, low survivability, the climate, the poaching, over-use, predators, diseases and low genetic diversity) for this, but there is no definite opinion on the issue. All researchers agree, however, that the reasons are complex. The decreasing number of brown hares has led to a change in their statute in the last decade. The species has been included in the Red List of the Endangered Species IUCIN in the category Least Concern (LC) (Smith, Johnston, 2008). The hare is listed in Application III of the Bern Convention (Vaughan et al., 2003, Smith et al., 2005). In Great Britain, it is classified as a 'priority species with conservation significance' (Smith et al., 2005), and Switzerland has included the species in its National Red List (Pfister et al., 2002).

Despite the declining tendency of the population in Bulgaria (Botev et al. 1985), the brown hare remains one of the most numerous local hunting species in the country. So much meat was obtained from it in the past that it was equal to the total remaining game. The yearly yield of brown hare skins (over 250 thousand pcs. in 1962) made up about 60 % from the total yield of game skins. (Ruskov, Petrov, 1968, Ruskov et al., 1972). During the years when its number exceeded 1 million individuals, the brown hare was a subject of trade. The peak in export was reached in 1968-1969 - over 40 thousand pcs. (Botev et al., 1985). The number of the brown hares in Bulgaria has been on a progressive decline from 1970 onward, but it has been more apparent since 1994. The official censuses report number within 370 to 420 thousand pcs. За последните 20 години броят на кафявия заек намалява до нивата на отстрел през 1970 г. After the high number and shooting from the 60s to the 80s, at present its number is at its lowest levels since 1952. Since 1995, the shooting has also began to decrease significantly, especially in 2003 (13 334 brown hares shot in total), when it reached its lowest point since 1933 (Zhelev, 2015; Zhelev et al., 2013). There are no official data about the shooting after 2009, but in 2012 and 2013, it did not exceed more than 5 thousand brown hares yearly (Zhelev, 2015).

Hunting of brown hares during 2012 occurred in 54 Hunting and Fishing Units (HFU) in the country, out of a total of 140 (HFU) in the structures of the National Association of Hunters and Anglerism - NAHA (without the members of Bulgarian Union of Hunters and Anglers - BUHA). In 2013, there were already 48 HFU, where 6 HFU free willingly denied themselves from hunting (Zhelev, 2015).

Studies about the reasons leading to the low number in the whole country have not been made in the last 30 years. A research on reproduction was made to deter-

mine the reasons for the decreasing hare population. We research the testicle weight of the European brown hares from Bulgaria of various ages, obtained from different months of the year in order to get an overview of the normal sequences of the morphological changes in the gonads during the year.

## Materials and methods

For the time period from 2009 to 2014, 315 European brown hares form different ages underwent year-long testes weight measurements, and testicular activity was studied (Table 1 and Table 2). The samples were collected from 121 hunting grounds around the country under 600 m a.s.l. From all testes, 55 pcs were taken outside of the hunting season, in the period January – September, from 43 hunting grounds. The IBM SPSS Statistics, 19 (2010) (IBM corporation) software was used for the calculation of t-test at the various analyses. All weights were measured with an electronic scale Sartorius L 1000S, with an accuracy of up to 0.01 g. Their diameter was measured with a digital gauge IDF, L – 300 mm, with an accuracy of up to 0.1 mm. The age was defined according to the weight of the eye lens, described by Suchentrunk et al. (1991) and also from skull characteristics (Cabon-Raczynska, 1964; Pepin, 1973; Suchentrunk, Davidovic, 2004) and the Stroh (1931) method. Four classes of age for juvenile hares (up to 1 year of age) were identified regardless of the fact, that some of them were born at the end of the previous year (1 – up to 3 months, 2- from 4 to 6 months, 3 – from 7 to 9 months and 4 – from 10 to 12 months). Three classes were defined for adult brown hares (up to 1 year of age): 5 – from 1 to 2 years, 6 – from 2 to 3 years, and 7 – over 3 years. In order to compare the sequence on the testes weights distribution to the various ages with sperm density, we assigned sperm quantity (concentration of the spermatozoa) for each individual in the epididymis by binocular stereoscope (Carl Zeiss Germany) with a categorization into five classes by Hubenov (1974): 1 – no sperm, 2 – very little, 3 – moderately present, 4 – high amount, 5 – massive amount.

## Results and Discussion

We determine (Table 1) that there is a significant difference in the testes weights from age class 3 up to age class 7 between the periods (active and inactive). This is normal due to the fact that in the 7-th month the brown hare is already physiologically mature. We observe similar results with the sperm classes (Table 2), where the significant differences between the periods have already started at age class 2, with the exception of age class 3.

The results of a study by Lincoln et al. (1976) showed that juveniles reach puberty only during the breeding season of adults. The age at which puberty is reached varies with the date of birth. Males born before May reach puberty and become sexually mature at 2-3 months of age. Puberty is delayed for several months for those born

between May and July, and who reached physiological maturity in the autumn, in the inactive reproductive period. The third group, born after July, resemble those of the first group. They show strong reproductive tract growth at 3 months of age, which coincides with the start of the new breeding season. The delay of puberty in male hares is dictated by the oppressive photoperiodic conditions of autumn. The development of the reproductive tract begins at a normal age, but unfavourable environmental conditions delay sexual maturation until the start of the new breeding season.

The results show (Fig. 1) that the active reproductive season finishes at the end of August and the beginning of September, after which follows a period of calm when the testes weights were at their lowest. In September, October and the beginning of

**Table 1.** Testes weights distributed by age classes during the whole year for the research period 2009-2014 and statistical significance on the testes weight differences between the periods, compared with t-test

| Age class | *p* | Active reproductive period (January – September) | | | | | | | Inactive reproductive period (October – December) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Range | Min | Max | Mean | SE | SD | N | Range | Min | Max | Mean | SE | SD |
| 1 | 0.795 | 2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.0 | 0.1 | 6 | 1.8 | 0.2 | 2.0 | 0.5 | 0.3 | 0.7 |
| 2 | 0.222 | 3 | 0.3 | 0.3 | 0.6 | 0.4 | 0.1 | 0.1 | 26 | 5.4 | 0.3 | 5.7 | 1.1 | 0.2 | 1.2 |
| 3 | 0.009** | 8 | 13.3 | 0.9 | 14.1 | 7.3 | 1.6 | 4.6 | 71 | 7.9 | 0.3 | 8.3 | 3.2 | 0.3 | 2.2 |
| 4 | 0.010* | 7 | 14.7 | 1.7 | 16.3 | 8.7 | 1.6 | 4.3 | 30 | 9.6 | 1.2 | 10.8 | 3.9 | 0.4 | 2.3 |
| 5 | 0.000*** | 8 | 7.2 | 7.2 | 14.5 | 10.7 | 0.9 | 2.5 | 39 | 10.8 | 0.8 | 11.6 | 4.5 | 0.4 | 2.6 |
| 6 | 0.000*** | 25 | 13.0 | 1.7 | 14.7 | 9.7 | 0.8 | 3.8 | 81 | 10.0 | 1.4 | 11.4 | 4.7 | 0.3 | 2.4 |
| 7 | 0.020* | 2 | 2.8 | 10.4 | 13.2 | 11.8 | 1.4 | 2.0 | 7 | 19.6 | 3.6 | 23.2 | 9.0 | 2.5 | 6.6 |

Note: N – number individuals; statistical significance: *p < 0.05, ** p < 0.01, ***p < 0.001

**Table 2.** Testes weight distributed by sperm classes during the whole year for the research period 2009-2014 and statistical significance on the testes weight differences between the periods, compared with t-test

| Age class | *p* | Active reproductive period (January – September) | | | | | | | Inactive reproductive period (October – December) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | N | Range | Min | Max | Mean | SE | SD | N | Range | Min | Max | Mean | SE | SD |
| 1 | 0.557 | 2 | 0.4 | 0.2 | 0.6 | 0.4 | 0.2 | 0.3 | 23 | 4.7 | 0.2 | 5.0 | 2.2 | 0.3 | 1.5 |
| 2 | 0.019* | 5 | 1.0 | 0.1 | 1.1 | 0.6 | 0.2 | 0.4 | 84 | 6.7 | 0.2 | 6.8 | 2.2 | 0.2 | 1.4 |
| 3 | 0.068 | 9 | 8.7 | 1.7 | 10.4 | 5.9 | 1.2 | 3.7 | 117 | 11.3 | 0.2 | 11.4 | 4.0 | 0.2 | 2.2 |
| 4 | 0.022* | 26 | 8.7 | 6.0 | 14.7 | 10.2 | 0.5 | 2.5 | 22 | 20.7 | 2.5 | 23.2 | 7.9 | 0.9 | 4.0 |
| 5 | 0.002** | 13 | 9.6 | 6.7 | 16.3 | 11.6 | 0.9 | 3.2 | 14 | 8.0 | 3.1 | 11.1 | 7.7 | 0.6 | 2.2 |

Note: N – number individuals; statistical significance: *p < 0.05, ** p < 0.01, ***p < 0.001

November, the weights of the testes were low. The first indications for the start of the new reproductive season were shown in November, with the gradual increase of the testes weights. In January the testes weights reached high levels, as the highest weights were measured in May-June, with slightly lower values in July and August. After this follows a low weight spell, when the calm period starts in September, and the testes growth is reactive in December. Similar results are also recorded by Lincoln (1974), Blottner et al. (2000) and Simeunovic et al. (2000). All adult males (above 1 year old, n=160), had high significant values ($p<0.0001$) of testes weights during the whole year (see Table 1), compared to all the young ones (under 1 year old, n=155). The results showed a normal reproductive cycle, with periods of activity and calm in male European brown hares from Bulgaria.

Lincoln (1974) studied the reproductive physiology of 760 hares shot throughout the year to explain seasonal changes in behaviour and the so-called 'March Madness'. After the autumn rest period, the first indications of the new reproductive season appear in November, with the increase in weight and activity of the testicles. This trend continues from December to February. Reproduction in females begins most often in January with the first ovulation. In March and April, hares show full reproductive activity and almost all the females are pregnant, most often with three or more embryos. During these months ('March Madness') the breeding process is at its peak and many male hares are close to depleting their sperm reserves in the epididymis. In May, June and July, females continue to be highly fertile. In males, there is a decrease in the synthesis of testosterone in the testicles. The reproductive period declines and

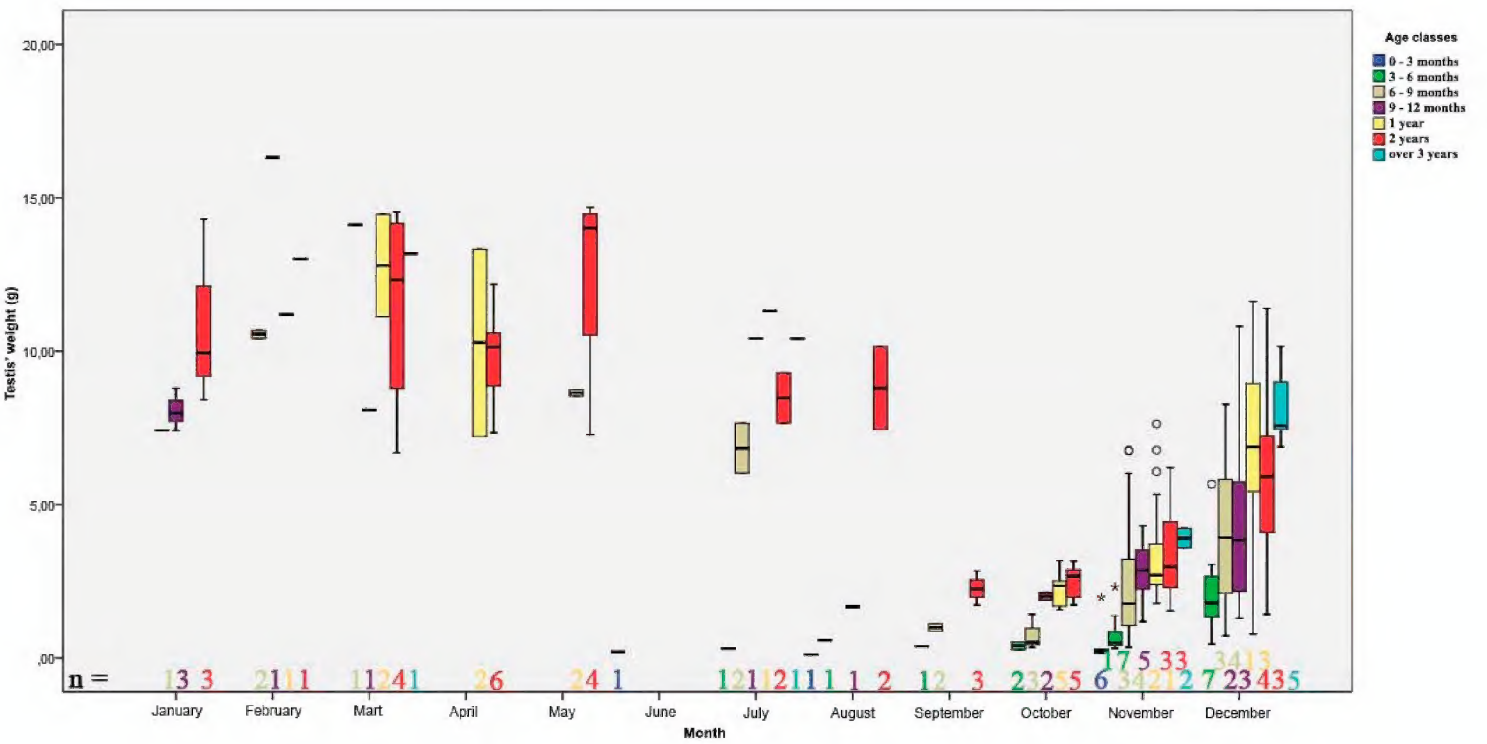


**Fig. 1.** Distribution of the testes weight from all individuals (n=315) during the whole year according to different age classes. For each class of age, coloured in different colours, the number of individuals belonging to it was also pointed bellow. Each month can have a maximum class of age seven (e. g. November) and minimum one (e. g. June), due to the lack of samples

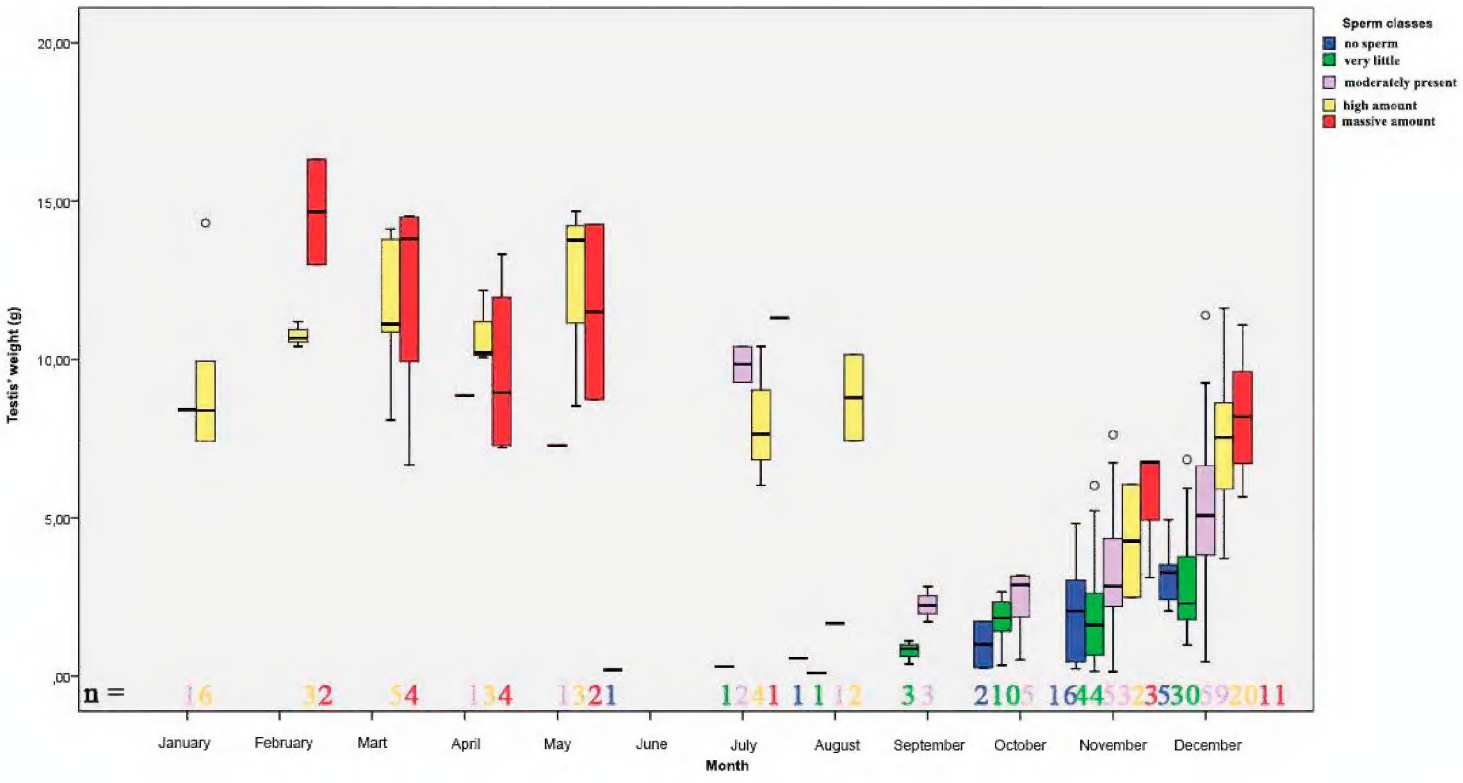


**Fig. 2.** Distribution of testes weight from all individuals (n=315) during the whole year according to different sperm classes. For each class of sperm, coloured in different colours, the number of individuals belonging to it was also pointed out below. Each month can have a maximum class of age seven (e. g. November) and minimum one (e. g. June), due to the lack of samples

ends in late July and early August. In the testes, there is a rapid regression in August and sperm production is constant (no peaks) until September. The sperm reserves in the epididymis are gradually depleted and the sperm count is reduced by October. In females, the number of births from one birth is also reduced and pregnant individuals are rare after September.

The distribution of testes weights, compared to the various ages, follows the same sequence as the sperm density. In view of the fact that the density of sperm is defined by the tail of the epididymis, and the weights were measured from the testes, the connection between them proves the existence of a normal reproductive cycle with stages of activity and standstill. The heaviest testes had the biggest quantity of sperm in the epididymis (Fig. 2), regardless of the season (see Table 2). We determined that the quantity of sperm and the weights of the testes were in direct dependency ($p<0.0001$), directly following the line of activity and standstill in the season, which confirms the results by Blottner et al. (2000, 2001).

The results related to the condition of the reproductive possibilities of hares in Bulgaria show that there is a normal sequence of the morphological changes in the gonads during the year. The reproduction of hares was completely normal, and the reproductive parameters were within the accepted ones for the areal of the species. That is why the notion that the probable reason for the low number is related to an impaired reproductive process can be dismissed with a high degree of certainty. Problems in reproduction were not observed, and the reason for the declining numbers of the species can be searched in other factors and reasons.

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